



Rapid Communication

## A Preliminary Investigation into the Morphology of Trabecular Bone Damage Associated with Intervertebral Disc Herniation

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### Abstract

Bone fragments have been identified in the material of many intervertebral disc herniations. The mechanism by which this occurs or exact source of these fragments remains to be determined. Further, the morphology of bone damage in the vertebral body before and after herniation has not been characterized. This study sought to determine the location and pattern of bone damage during intervertebral disc herniation. Porcine functional spinal units were imaged under micro computed tomography scans before and after repeated flexion and extension motions under compressive load. Presence of herniation was confirmed in all specimens. In addition to herniation, there was a characteristic decrease in trabecular bone density evident on high resolution imaging associated with trabecular micro-fracturing, directly beneath the endplate region of each vertebral body. The results indicate that the site of bone damage during intervertebral disc herniation is in a consistent location under controlled testing conditions, at least in the young adult porcine specimens tested here. Further, the region of damage is in a prime location for bone fragments to pass opportunistically through the endplate via any defect that may be pre-existing or acquired as a result of damage to the underlying trabecular scaffold. Future work should focus on examining and quantifying bone damage under a variety of loading scenarios to accommodate the variety of herniation and fracture types seen in vivo.

### Keywords

Intervertebral disc; Endplate; Herniation; Trabeculae; Vertebral body

### Introduction

Recent evidence has identified bone fragments in the harvested extruded material of many intervertebral disc herniations [1]. While there could be many plausible mechanisms through which this occurs given the variety of herniation types [2], identifying a repeatable mechanism for the morphology of bone damage during herniation is important in order to understand the etiology of this injury.

Previous research highlights the large number of endplate fractures that occur during the course of gradual disc prolapse [3] and acute prolapse [4]. As such, the endplate has been suggested as the weak-point of the intervertebral disc and the first structure that is

damaged in compression [5]. Specifically, work by Veres, Robertson, and Broom [6] characterized the interface between the endplate and annulus as a weak-point and region of failure. There has been no work however, that has characterized a repeatable pattern of damage to the vertebral body under controlled testing conditions. Clinically, herniations have been shown to resorb in some circumstances [7]. Herniated material containing only nucleus pulposus would be able to swell, rapidly lose proteoglycans, and shrink [8]. It is possible that the presence of bone or cartilage fragments could reduce such a shrinkage and would logically delay any reduction due to macrophage activity, providing a possible explanation into intensity and persistence of symptoms [9].

Many questions remain regarding the origin of bone fragments in herniated material: such as whether the origin is from a predictable location, the morphology of this form of bony injury, and implications for further injury. Characterizing the pattern, location, and repeatability of bone damage to the endplate during herniation is an important first step in beginning to systematically identify the mechanism behind the presence of bone fragments within herniated material.

The purpose of this investigation was to characterize and identify the morphology of bone damage during disc herniation under controlled testing parameters.

### Materials and Methods

#### Specimens and preparation

Six porcine cervical spines (mean age: 6 months, weight: 80 kg) dissected into motion segments consisting of the C5 and C6 vertebral bodies and the intervening disc were used for this investigation. A further two motion segments, one consisting of the C3 and C4 vertebral bodies and intervening disc and the other consisting of the C5 and C6 vertebral bodies and intervening discs served as controls. Specimens were stored frozen and then thawed for a period of 12 hours prior to dissection. During dissection, muscular tissue was removed from the motion segments while keeping ligaments intact. At this stage, specimens were injected with a radio-opaque dye consisting of a 2:1:2 composition of omnipaque, blue dye (Coomassie brilliant blue Gmix 0.25% dye; 2.5% MeOH; and 97.25% distilled water), and distilled water. This was inserted through the anterior disc using an 18 gauge needle. It was injected up until the point where the disc could not passively contain any more dye. The mixture was of a consistency where it would not diffuse through the annulus unless a fissure was present [10]. Use of this dye facilitated tracking of a herniation via radiological imaging and confirmed the presence of an intervertebral disc herniation. After initial dissection specimens were labeled and frozen for transport to another facility for micro computed tomography (micro-CT) imaging.

Motion segments were returned after imaging and mounted and secured in stainless steel cups using screws inserted through the endplates of the superior and inferior vertebral bodies, 18-gauge wire looped bilaterally around the lamina and anterior processes, and filling the cups with non-exothermic dental stone (Denstone<sup>®</sup>, Miles, South Bend, IN, USA). A saline-soaked cloth and plastic

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wrap surrounding the specimens maintained hydration throughout testing. Control specimens underwent this mounting process but were not subject to the mechanical testing protocol. This was done to ensure that the mounting process did not produce any damage to the vertebral bodies.

### Medical imaging

High resolution imaging was performed on all tested and control specimens using a GE eXplore speCZT micro-CT (GE Healthcare, Division of General Electric Company) with scanning parameters of 90 kilovolts x-ray tube voltage and tube current of 40 milliamps. Isotropic voxel size of 0.050 mm (50 micrometers) was achieved with 3-dimensional image reconstruction performed at 50 and 100  $\mu$ m. The 3D datasets for each specimen were exported in DICOM (Digital Imaging and Communications in Medicine) format to Advanced Visualization software (AW Workstation, GE Healthcare) for analysis.

### Testing protocol

Mounted specimens were placed in a dynamic servohydraulic testing apparatus (Model 8511, Instron Canada, Burlington, Ontario, Canada). The bottom portion of the mounting apparatus was able to translate freely on a surface of ball bearings with flexion and extension motions being created by an electric brushless servo-motor (model BNR3018D, Cleveland Machine Controls, Billerica, MA, USA) and planetary gear head (model 34PL0400, Applied Motion Products, Watsonville, CA, USA). A customized software interface controlled the system.

Prior to testing, each specimen was loaded to 300N of axial compression for 15 minutes to remove any post-mortem swelling that may have occurred [11]. Specimens were then subject to a 1500N compressive load and a passive test, establishing the linear region of angular displacement versus axial torque similar to the neutral zone described by Panjabi et al. [12], this determined the angular displacements to be used for each specimen.

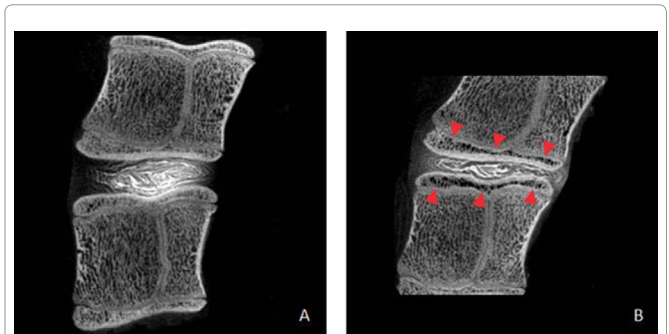
Specimens were then exposed to 10000 cycles of repeated flexion and extension motions at 1Hz under 1500N of axial compression. After mechanical testing was completed, specimens were immediately re-frozen for transport and re-imaged under micro-CT. After imaging, several experimental specimens including one control were imaged under microscopy to serve as representative samples of the effects of the testing protocol.

### Data analysis

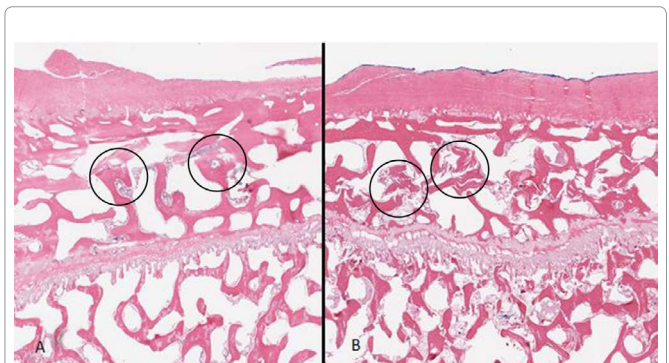
The micro-CT images of the tested and control specimens were interpreted by a radiology resident and a staff neuroradiologist. Descriptions of the presence and location of osseous tissue damage in addition to the presence of an intervertebral disc herniation was provided.

### Results

Evidence of intervertebral disc herniation was present in all specimens that were mechanically tested. No disc herniations were identified in any control specimens. Micro CT images of the test specimens were reviewed in high contrast to accentuate trabecular detail. Review of the images from the tested specimens revealed sub-cortical lucent regions (Figure 1) consistent with gas density and evidence of decreased trabecular density immediately deep to the vertebral endplates to a variable degree in all tested specimens. This phenomenon was not observed in control specimens that underwent



**Figure 1:** (A) Porcine spine specimen prior to mechanical testing. (B) Same specimen after undergoing a herniation protocol, note the distinct regions directly beneath the endplate where the trabecular bone appears to have fractured and the density reduced. Arrowheads denote the boundaries of trabecular bone damage.



**Figure 2:** (A) Microscopic image slice of a control specimen in the area directly beneath the endplate. Normal regions are circled for comparison with abnormal regions in (b). (B) Microscopic image slice of a fatigued specimen where there is evidence of fracturing and buckling of the trabecular bone not seen in the control specimen. Selected abnormal buckled regions are circled in order to highlight the differences between the undamaged specimen in (a) and the damaged specimen here.

the mounting procedure but were not subject to mechanical testing. Tested specimens that underwent microscopy demonstrated sub-endplate trabecular fractures (Figure 2).

### Discussion

Evidence from the micro-CT images assessed in this study characterizes a consistent pattern of sub-endplate damage across all specimens. With a moderate compressive load and repeated flexion/extension motions, the nucleus pulposus herniated through the annulus while trabecular bone experienced fracturing and disorganization directly beneath the endplate. These results agree with previous findings [1] and provide a plausible location and mechanism for the origin of bone fragments in herniated material. A small crack in the endplate, Schmorl's node, or avulsion fracture would provide an adequate conduit for this material. These preliminary findings suggest that the mechanism by which bone is damaged during herniation is a predictable and repeatable process.

Future work which tests a variety of other loading and repetitive cycle schemes will determine the morphology of bone damage across a variety of herniation scenarios. Herniations containing bone or cartilage present a risk of being more complex and resisting being

resorbed [13]; this could result in a persistence of symptoms [9]. While not assessed or examined in this study, small bone fragments could find themselves present in herniated material by passing through the endplate via a structural defect. This is a plausible scenario given the documentation of endplate fractures shown to occur during herniation [3,4]. Results from this study agree with work done by Holmes and Hukins [14], identifying sub-endplate trabecular damage after loading cadaveric tissue in pure compression. The current study used a similar compressive load but added dynamic flexion and extension motions to produce a posterior disc herniation accompanying the trabecular bone damage.

This study was limited primarily in its diversity of load testing. Only one value of axial compression was used and results were not obtained for trabecular bone damage at different levels of axial compression used for fatigue testing. We can surmise that with lower compressive loads, trabecular bone damage would be lower while with higher compressive loads, trabecular bone damage would increase. The compressive load that specimens were subject to in this study can be likened to the load and coupled bending, experienced by the lumbar spine during a light lifting task [15]. This study was also limited in its quantification of trabecular bone damage. Qualitative data are presented here which describe a characteristic pattern of damage that can be anticipated given a similar injury mechanism to another spine. While quantification would be useful in the case of comparing various loads, as a preliminary proof of principle study, the qualitative analysis is sufficient. This work also presents an ex-vivo scenario of tissue loading and does not take into account any adaptive tissue remodeling that would occur in-vivo. In cases where there is significant overload or insufficient time for tissue remodeling and repair however, the results seen in this study could indeed highlight a mechanism for what potentially occurs in-vivo.

Use of an animal model presents a limitation to the current study and its potential extrapolation to results seen across a population of humans. Previous work does indicate that the porcine cervical spine is a suitable analog to the human lumbar spine with respect to anatomy, geometry [16], and function [17], with perhaps the closest comparison to the lumbar spine of a human adolescent [18]. Use of a porcine cervical spine also has the advantage of providing greater experimental control for factors such as age, weight, activity, diet, and genetics; this facilitated specimen homogeneity, ensuring that other extraneous variables would not account for the results seen.

Findings from this study provide proof of principle evidence under controlled testing conditions that the site of bone damage associated with intervertebral disc herniation lies directly beneath the endplate. This provides a pathway to pass bone and endplate fragments through the endplate as they are stripped away [13] to join herniated material extruding from the disc. This could potentially create more complex herniations that do not resorb [13] and result in persistence of painful symptoms. Future work should seek to examine the morphology of this bone damage under a greater variety of herniation scenarios and begin to numerically quantify and assay the extent of damage and quantity of bone in herniated material.

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